6.10 Biological Sampling Procedures

6.10.1 Phytoplankton Sampling

6.10.1.1 Sample Site Location

Locate sampling stations as near as possible to those selected for chemical and bacteriological sampling to ensure maximum correlation of findings. These locations will depend upon the physical nature of the water body. In streams or rivers, stations should be established both upstream and downstream of a pollution source or major tributary. Stations should also be set up on either side of the river so as to account for unequal lateral mixing. Slow moving sections of streams generally contain more phytoplankton than slower moving segments. If there are any lakes, reservoirs, or backwater areas (i.e., potential phytoplanton sources) upstream of sampling stations, notes on their nature and location should be included in the sampling log.

Sampling stations in lakes, reservoirs, estuaries and the ocean should be located along grid networks or transect lines, aligned so as to provide the most representative sampling. Points of interest should include intake and discharge areas, constrictions within the water body, and major bays and tributaries off of the main basin. In tidal areas, the effects of tidal oscillation should also be taken into account when determining sampling frequency. When locating stations for a *red tide* survey in estuarine or coastal waters, note where and when the blooms tend to occur.

6.10.1.2 Sampling Depth

Rivers, streams, shallow bays and coastal waters are usually well mixed so that only subsurface sampling is necessary. In lakes, reservoirs, as well as deeper coastal waters, plankton composition and density may vary with depth; thus sampling should be done at several depths determined by the depth of the thermocline, the euphotic zone if applicable, and overall the depth at the station. In shallow areas (1-2 meters) subsurface samples (to a depth of 1M) are usually sufficient. In deeper lakes and reservoirs, samples should be taken at intervals of 5M or less to the thermocline. In estuarine and coastal waters 2-10M deep, subsuface, mid-depth and near bottom samples are recommended. Offshore samples should be collected at intervals of 5M or less to the bottom of the thermocline, and near the bottom where depletion of oxygen by decaying blooms is critical; larger sample volumes of at least one liter are needed because these waters are typically low in productivity.

6.10.1.3 Sampling Procedure

Sample size, preservation and storage are dependent upon certain variables. Refer to Chapter 2, *Appendix 2.1* for details.

If analysis is limited to species composition clear polyethylene or glass bottles may be used. If chlorophyll analyses is requested, amber bottles are recommended. Clear or translucent glass or plastic bottles may be used provided they are covered with aluminum foil so as to shield out light.

Freshwater samples for species composition analysis should be preserved with a solution of neutralized formalin (5 ml neutralized buffer with formalin/100 ml of sample). Estuarine and marine samples are to be preserved with Lugol's solution (60 g KI + 40 g iodine crystals in 1,000 ml distilled water) at a rate of one (1) drop Lugol's solution to 100 ml of sample adding more periodically to maintain the color of weak tea. In special studies glutaraldehyde may be used (6-drops/25 ml of sample). All preserved samples should be stored in the dark immedi-

ately so as to prevent the degradation of the phytoplankton, or the preservative if Lugol's solution is used.

All species composition phytoplankton samples should be fixed (preserved) except where primary productivity and phytoplankton populations must be studied in extensive detail. When collecting live samples, leave at least a four-cm air space in the bottle and chill to 4 ºC (e.g. in a cooler with ice) during transit storage. For delicate flagellated species do not refrigerate sample bottles. Maintain in-situ temperature by storing them out of direct sunlight, in an ice chest, with some of the ambient water. Surface samples in streams, rivers, shallow estuaries and coastal water can be collected simply by inverting the sample bottle, immersing it up to one (1) meter below the water surface and slowly filling it as it is removed from the water. A Kemmerer sampler may also be used, holding it in a horizontal position and closing it manually.

Samples collected for Chlorophyll analysis shall not be fixed preserved. Chlorophyll samples shall be preserved by chilling to 4°C. If species composition analysis is necessary, then it shall be collected in a separate sample bottle, or fixed preserved by laboratory staff after the aliquot for chlorophyll analysis is removed from the sample container.

When deeper samples are needed, use of a Kemmerer, Water Bottle, Van Dorn or Juday samplers are standard. All of these devices basically consist of a metal or plastic hollow cylinder with remotely activated stoppers at either end. The sampler is lowered to a desired depth with a graduated line. Once at the desired depth, a heavy brass slug or messenger, attached to the line, is released. It slides down the line, strikes the release mechanism on the sampler which pulls the stoppers tight against the open ends of the cylinder, trapping the sample of water inside. The sampler is then withdrawn and the water emptied into the sample container via a small spigot or tube in one of the stoppers. Use only non-metallic samplers when metal analysis, algal assays, or primary productivity measurements will be performed on the sample.

Sample bottle labels should identify the body of water sampled and list the date of collection, collectors name, preservative if present, and the type of biological analysis desired (determination of dominant or bloom species, total cell count, etc). It is important that labels clearly identify live plankton samples as being unpreserved.

6.10.2 Zooplankton Sampling

6.10.2.1 Sample Site Location

The procedures outlined for phytoplankton sampling can be applied.

6.10.2.2 Sample Depth

The same procedure as phytoplankton for rivers and streams but in lentic environments sample at one (1) meter intervals from the surface to the lake bottom; since these organisms are not confined to the euphotic zone.

6.10.2.3 Sampling Procedure

Zooplankton analysis requires larger volume samples than phytoplankton, at least six (6) liters in moderately and highly productive waters. For appropriate preservation requirements refer to Appendix-A.

6.10.3 Macrophyte Sampling

Field observations are very important when analyzing macrophyte populations. The sampling person must estimate the percentage of the lake's surface area, and bottom area if possible, over

which macrophyte growth occurs and the dominant form or forms for any samples taken.

When taking a macrophyte sample, an entire plant of each kind encountered should be collected if at all possible. If this is not possible, as much of the plant as can be collected should be taken, and care should be taken to include any reproductive structures present, complete leaves, and a section of stem showing branching pattern, if any. Specimens can be placed in plastic bags or containers without special preservatives, although completely aquatic species should be kept moist; refrigeration is recommended unless otherwise specified. If the samples cannot be examined within 3 days, it is recommended that they be preserved with a 5% solution of formalin.

6.10.4 Macroinvertebrates

6.10.4.1 Hester-Dendy Artificial Substrates

6.10.4.1.1 Sampler Placement

These multiple-plate samplers consist of eight large tempered plates separated by seven small plates, exposing one square foot of surface area. A hole is bored through the center of each plate. Plates placed alternatively on a galvanized eyebolt, threaded rod or nylon cord and secured. Samplers may have a brick attached to one end to anchor the sampler to the bottom for use in shallow streams, or they may be suspended from anchored floats in lakes and deep rivers. Used throughout, artificial substrates provide consistency of habitat in order to facilitate comparison among stations. Samplers are usually placed at equal intervals across a stream. However, species colonization is greatly affected by current velocity. When conducting a survey, care should be taken to place substrates at locations having similar flow characteristics. Three samplers are routinely placed at each sample site, although more samples may be necessary to satisfy particular statistical criteria. When using brick-anchored samplers, additional rocks are often necessary to secure the sampler in an upright position. Care should be taken not to block the plates with the rocks and thus limit colonization. Sampling devices should be placed as inconspicuously as possible, since they are prone to removal by the public. They should be secured with strong nylon line (not attached to the anchor line itself). In deeper waters, suspended samplers should be placed within the euphotic zone (i.e., shallower depths where light penetrates) usually less than 2 meters.

6.10.4.1.2 Sampler Retrieval

The samplers should be removed after a six-week colonization period. Gently remove the sampler from the water in order not to dislodge the organisms, and immediately place the sampler in a plastic tub or bucket. Anchors attached to the substrate should not be placed in the tub until any organisms on the anchor are removed and discarded. Add a small amount of water to the tub and wash the easily removable material from the plates. Then gently scrape the top and bottom of each plate into the tub removing the plates as cleaned. Scalpel, spatula or soft toothbrushes are useful cleaning tools. Pour the sample slurry through an U.S. Standard No. 30 sieve. Additional water may be used to completely clean the tub. Pass this through the sieve as previously described. Transfer the sample material from the sieve to the sample jar(s) using forceps or a stream of water from a wash bottle. Fill each jar no more than half full. Work directly over the tub so that any spilled materials can be recovered. Finally, inspect the tub for any remaining organisms and transfer them to the sample jar(s).

Water-resistant paper should be used for sample labels and all information written with a soft lead pencil. Include sample (log) number, water body, station, sample number, sample device, and other pertinent information. Record the sample number in a bound notebook together with other environmental information. Place the label inside the sample jar. An external label is helpful in identifying the sample in the laboratory. See below for preservation. Any samplers thought to be contaminated by oil, grease, toxins, etc. should not be reused. All other samplers are to be washed thoroughly in the laboratory before reuse.

6.10.4.2 Surber or Square Foot Bottom Sampler

6.10.4.2.1 Sampler Placement

This sampler consists of a strong close-woven fabric (0.595-mm opening) approximately 69-cm (27 in.) long held open by a square foot metal frame hinged at one side to another frame of equal size. The sampler is generally used in procuring samples in fast-flowing streams less than 1m deep. It can also be used in pools where the water depth is wadeable. Three replicate samples are usually obtained at each sampling station.

Carefully place the sampler in position with the net opening facing upstream, using the current to hold the net open, while standing downstream and to the side of the sampling area. By imbedding the separate 2 or 4-inch extensions of the horizontal frame, the sampled area will be more effectively isolated. When taking replicate samples, always work across or in an upstream direction. Dislodge the rocks, stones, and other bottom material within the frame to a depth of at least 2 inches and collect them in the net.

6.10.4.2.2 Sampler Retrieval

Remove the sampler and empty the contents into a plastic tub. Carefully inspect the larger rocks and stones removing any organisms clinging to them, and discard the stones when cleaned. Also carefully inspect the net and remove any organisms remaining. After the larger materials have been inspected and removed, add a small amount of water to the tub and pour the slurry through an U.S. Standard No. 30 sieve. This may have to be repeated several times in order to completely empty the tub. Follow the same techniques described under Hester-Dendy retrievals in transferring the sample to the sample jars and in labeling. See below for preservation.

6.10.5 Grab Samplers

The Ponar, Peterson, and Ekman grab are the most commonly used grab samplers. The Ponar is similar to the Peterson, except that it has side plates and a screened top to prevent sampling loss. The Ekman grab is useful in sampling silt and muck in water with little current. Extreme care must be employed when locking open the jaws of the samplers, as premature tripping will squash or sever fingers or hands. Handling by the attached line is recommended with an open sampler. Carefully lower the grab to the bottom so as not to agitate the substrate prior to sampling. Slacken the rope to trip jaws (the Ekman grab employs a messenger, which is released by the operator) and retrieve the sampler. Place it in a plastic tub or large screened bin and carefully open the sampler jaws to release the sample. The sample should be discarded if sticks or stones have obstructed the jaws or if there is incomplete closure for any other reason. Inspect the larger debris for organisms and discard the debris when cleaned. Filter sample through a #30 sieve to remove smaller particles. Then transfer, label and preserve the sample as described in Chapter 2, *Appendix 2.1*.

A Mason jar, or any glass or plastic wide mouth container can be used for macroinvertebrate samples. All macroinvertebrates are preserved in 5% formalin (5 ml formalin/100 ml of water from which the organism was taken), with 95 % ethanol, or isopropyl alcohol.

Equipment List for Macroinvertebrate Sampling Using Surber, Square-Foot, Hester-Dendy or Grab Samplers

- U.S. Standard No. 30 Sieve
- Plastic Trays
- Brush
- Forceps
- Gloves
- Mason Jars
- Boots
- Formalin
- Labels
- Squeeze Bottle

6.10.6 Periphyton Sampling

6.10.6.1 Artificial Substrates

6.10.6.1.1 Sampler Placement

Samples are collected using standard 25 x 75 mm (1 x 3in) unfrosted glass microscope slides as artificial substrates mounted in a floating rack. Eight slides are to be placed at equal intervals in the sampler and secured with monofilament fishing line. The sampler is then attached several feet downstream of a large anchored float. The sampler should be secured so that the slides are parallel with the current. The large float helps to deflect floating materials, which would otherwise cover the slides and reduce photosynthesis. It also forms an eddy, which may be more conducive for periphyton colonization than a faster current. In shallow streams, the sampler may be tied directly to a brick and placed directly on the stream bottom. This is especially advantageous in areas where floating samples may be disturbed or removed by the curious. Care should also be taken to place the samples in well lighted stream segments so that light intensity will be similar at all stations in a survey.

6.10.6.1.2 Sampler Retrieval

A two-week exposure period constitutes the optimum exposure period. Upon retrieval, three slides should be immediately processed for chlorophyll A determinations. If it is impossible to begin immediately (while rowing a boat for example) place the sampler in a bucket or tub and cover, since exposing the slides to direct sunlight will result in a rapid deterioration of chlorophyll.

To process chlorophyll, scrape three slides clean as soon as possible with a razor blade or rubber policeman, being careful not to touch the surfaces with your fingers. Place the scrapings from each slide into separate 120 ml amber jars (with polyseal caps) and then, using an eyedropper, rinse each slide with a small amount of 90% acetone. Twenty to thirty milliliters to a maximum of fifty milliliters should suffice. The remaining slides, to be used for species composition determination, should be placed in separate clear glass jars filled with 5% formalin.

Seal jars tightly and label appropriately including station, sample number, date, and collector's name. Place samples in an ice chest for transport to the laboratory. Process the slides used for chlorophyll analysis (and later, ash-free weight) first since chlorophyll degrades rapidly and, if a slide is broken or contaminated the extra slide can be substituted.

Equipment List for Placement and Retrieval of Diatometers for Periphyton Sampling

- Boots
- Knife
- Labels
- Gloves
- Bricks
- String
- Diatometers
- Plain Glass Slides
- Nylon Monofilament
- Wide Mouth Amber Bottles
- Razor Blades or Rubber Policemen
- 90% Acetone (for chlorophyll A samples)
- 5% Formalin (for taxonomic ID samples)

6.10.6.2 Natural Substrates

If differences between substrates at the various study stations are not great, it is often advantageous to sample the natural substrates available. To do this a rubber sheet with a 10-cm2 space cut out of its center is placed on a rock, piece of wood or large plant stem or leaf taken from the water. A small amount (about 1 ml) of acetone solution (90% acetone, 10% distilled water) is sprayed on the area exposed by the cut out section of the rubber sheet. This area is then scrubbed with a toothbrush, which is repeatedly rinsed off with the acetone solution into an amber jar. The scrubbing and rinsing continues until the exposed area of substrate and toothbrush are clean. Approximately 20-30 ml of acetone solution is needed per sample.

For chlorophyll and ash-free weight determinations, 3 replicates per station are required, each taken from a separate substrate unit (e.g., 3 separate rocks or logs). For species composition analysis, substitute water for acetone and add enough formalin to the sample jar to make a 5% solution. One composite sample should be sufficient, made from scrapings from each of the substrates used for chlorophyll sampling. Label all jars with the station designation, date, preservative used, area of substrate cleaned, and operation to be performed.

6.10.7 Rapid Bioassessment (RBP) Techniques*

Rapid bioassessment provides an efficient tool for screening, site ranking and trend monitoring regarding quality of the State's waters. The methods currently in use pertain to lotic waters (i.e., streams and rivers). * from USEPA 1999, *Rapid Bioassessment Protocols for Use in Streams and Rivers*, Second Edition. EPA 841-B-99-002. Washington, D.C.

6.10.7.1 Benthic Macroinvertebrates

Benthic RBPs usually employ direct sampling of natural substrates, as do Surbers and grab samplers; under certain conditions, however, such as in large rivers, the use of artificial substrates may be more appropriate for RBP analysis. The collection procedure should provide

representative samples of the macroinvertebrate fauna from comparable habitat (substrate) types at all stations in a particular survey. Either single or multiple habitat samples can be employed depending on which is more suitable for a particular survey. A riffle/run habitat, with rock substrate, will generally provide the most diverse community of major macroinvertebrate groups. If the stream or river is non-wadeable or has an unstable substrate, fixed structures (e.g., submerged boulders, logs, bridges, and pilings) can provide suitable habitat.

D-framed or rectangular framed, 500 – 900 mm mesh "kick" nets can be employed as either single or multiple habitat samplers.

6.10.7.2 Single Habitat Sampling

A 100 m reach representative of the characteristics of the stream is chosen, and whenever possible, upstream of road or bridge crossing.

A composite sample is taken from individual sampling spots in the riffles and runs in the stream reach. A minimum of $2m^2$ composited area is sampled.

Sampling begins at the downstream end of the reach and proceeds upstream. 2 to 3 *kicks* are sampled at various velocities in the reach. A *kick* is a stationary sampling accomplished by positioning the net on the bottom of the stream and disturbing one square meter upstream of the net. The substrate can be disturbed using the heel and toe of the boot, or rubbed by hand for larger substrate particles. Several kicks will make up the composite sample.

Empty the composite sample into a sieve or sieve bucket and mix to ensure a homogeneous composite. Place the sample into a sample jar of at least one liter, and label with the site name, location, date, and sampler(s) name. Preserve the sample with 95 % ethanol, or isopropyl alcohol, or 5 % formaldehyde.

6.10.7.3 Multi-habitat Sampling

For sampling low gradient streams or streams with variable habitats, a multi-habitat sampling approach is required.

A 100 m reach representative of the characteristics of the stream is chosen, and whenever possible, upstream of road or bridge crossing.

Sampling begins at the downstream end of the reach and proceeds upstream. Habitats are sampled in their approximate proportion to their representation of surface area in the reach. In low gradient streams, snags, vegetated banks, submerged macrophytes, and gravel/ sand are habitats that support fauna. A total of 20 *jabs* or *kicks* should be sampled over the length of the reach. A *kick* is a stationary sampling accomplished by positioning the net on the bottom of the stream and disturbing one square meter upstream of the net. The substrate can be disturbed using the heel and toe of the boot, or rubbed by hand for larger substrate particles. A *jab* consists of forcefully thrusting the net into a productive habitat for a linear distance of 0.5 m. Then, sweep the area with a net to ensure macroinvertebrates, that have disengaged from the substrate, are collected. A minimum of 2 m^2 composited area is sampled

Empty the composite sample into a sieve or sieve bucket and mix to ensure a homogeneous composite. Place the sample into a sample jar of at least one liter, and label with the site name, location, date, and sampler(s) name. Preserve the sample with 95 % ethanol, or isopropyl alcohol, or 5 % formaldehyde.

6.10.7.4 Periphyton

Benthic algae (periphyton) are primary producers and important foundation of many stream food webs. Periphyton also stabilize substrata and serve as habitat for many other organisms. Their characteristics are affected by physical, chemical, and biological disturbances that may occur in the stream reach.

Equipment:

- stainless steel teaspoon, toothbrush, or similar brushing and scraping tools.
- section of 3" diameter or larger PVC pipe fitted with a rubber collar at one end
- white plastic or enamel pan
- petri dish and spatula
- forceps, suction bulb, and disposable pipets
- DI water
- 125 ml wide mouth sample jars
- labels
- preservative (Lugol's solution, 4% buffered formalin, "M3" fixative, or 2% glutaraldehyde)
- cooler with ice

Establish the sampling reach as per benthic macroinvertebrates above

Collect samples using techniques for specific substrate types:

Removable substrates (hard): gravel, pebbles, cobble, and woody debris. – Remove representative substrates form the water; brush or scrape a representative area of algae from the surface and rinse into sample jar.

Removable substrates (soft): mosses, macroalgae, vascular plants, root masses. – Place a portion of the plant in a sample container with some water. Shake it vigorously and rub gently to remove algae. Remove plant form sample container.

Large substrates (not removable): boulders, bedrock, logs, trees, and roots. - Place PVC pipe with a neoprene collar at one end on the substrate so that the collar is sealed against the substrate. Dislodge algae in the pipe with a toothbrush, or scraper. Remove algae from pipe with pipette.

Loose sediments: sand, silt, fine particulate organic matter, clay. – Invert petri dish over sediments. Trap sediments in petri dish by inserting spatula under dish. Remove sediments from stream and rinse into sampling container. Algal samples from depositional habitats can also be collected with spoons, forceps, or pipet.

Place samples collected from all substrate types into a single watertight, unbreakable, wide mouth container. If a single habitat is sampled, collect from several areas. A composite sample measuring four ounces (125 ml) is sufficient. Add preservative, and place label on outside of container with pertinent information.

Transport samples on ice and in the dark.

6.11 Toxicological Sampling (Toxicity Test or Bioassay)

6.11.1 Dilution Water Sample Collection and Handling:

Dilution water is acceptable for use in a bioassay provided healthy test organisms survive in it through the acclimation period without showing any signs of stress, including but not limited to, abnormal behavior or discoloration.

Dilution water samples shall be representative of the receiving water system which the effluent is discharged into. Samples shall be collected in the following manner:

In non-tidal waters, dilution water samples shall be collected from a location as close as possible to, but upstream of, the effluent-mixing zone.

In estuarine waters, dilution water samples shall be collected from a location as close as possible to, but upstream of, the effluent mixing zone. Samples shall also be collected during the outgoing tide up to and during low slack tide.

In marine waters (that is, tidal saltwater), dilution water samples shall be collected from a location outside the influence of the effluent being tested.

The sampling location shall be such that the salinity of the sample shall be within the salinity range for receiving water immediately outside of the effluent mixing zone.

When samples are collected from streams or rivers, an integrated sample shall be collected. This is a sample that is collected from bottom to top of the water column so that the sample collected is proportional to flow. If only a grab sample can be taken it should be collected at mid-depth in midstream.

When samples are collected from reservoirs or lakes, the effects of seasonal stratification, runoff, and previous rainfall upon the chemical/physical characteristics of the water shall be considered.

If the receiving water has a natural pH below 5.0 units, then the dilution water samples shall be adjusted to pH 5.0 prior to their use in test organism acclimation and/or toxicity test.

If the receiving water is influenced immediately upstream of the effluent outfall by other point sources of pollution so as to disqualify its use as dilution water, (in accordance with the NJPDES permit), then the dilution water sample(s) shall be obtained from a location just above the other point sources in the case of streams, or outside the zone of influence of other point sources in the case of other water bodies.

If acceptable dilution water cannot be obtained from the receiving water at any location because an effluent is discharged into the receiving water headwaters, then some other unpolluted water, meeting the following requirements, shall be used as an alternate in the following order of preference:

Another surface water or ground water having a natural quality similar to that of the receiving water prior to its pollution may be used; or

Reconstituted or artificial freshwater or saltwater having a natural quality similar to that of the receiving water prior to its pollution may be used; and

Substitute dilution water shall have a total hardness, total alkalinity, salinity and specific conductance within 25 percent and a pH within 0.4 units of the receiving water prior to its pollution, but not less than 5.0 units.

Alteration of dilution water samples shall be limited to the following:

Filtration is conducted through screening made of a non-toxic material. This screening shall have a mesh of 2 mm or larger if sample is to be used for fish testing or 0.45 microns or larger for zooplankton and macrocrustacean testing.

Adjustment of the salinity of dilution water samples shall only by either the addition of laboratory pure water to lower the salinity or by the addition either a hypersaline brine or artificial sea salts to raise the salinity made in accordance with N.J.A.C. 7:18 9.5(a)6.

Sample collection and transport containers shall meet the requirements listed in Appendix 3-1. Prior to sample collection, containers shall be pre-rinsed with the dilution water and then filled so that there is little or no air in the container neck or cap.

Dilution water sample storage shall be in covered containers constructed of non-toxic materials as specified in N.J.A.C. 7:18-7.3(a)13.

Dilution water samples shall not be stored for more than 150 hours and should be collected as close as possible to the time of testing.

6.11.2 Effluent Samples Shall be Collected and Handled in the Following Manner.

Unless otherwise specified by the Department, the effluent sampling location shall be the same as that specified in the applicable permit. The Department may specify an alternate sampling location when the following conditions prevail:

- When there is better access to the effluent at a point located between the final treatment and the discharge outfall. That point shall be the sampling point, or
- When the chlorinated effluent is dechlorinated prior to discharge and the purpose of the test is to determine the toxicity levels of the dechlorinated effluent. The sampling point shall be located after dechlorination.

The following sampling procedures shall be adhered in order to insure a representative effluent sample:

If the facility discharges wastewater continuously, the following procedures shall be used: Twenty-four hour composite samples consisting of equal volumes collected at least once every hour or a flow proportionate 24 hour composite sample shall be collected and used to set up a single toxicity test. This procedure is repeated for the duration of toxicity tests or; the effluent shall be pumped directly and continuously into the dilutor system of the toxicity test, for the duration of the test.

If the facility discharges wastewater intermittently, one of the following procedures shall be used:

When the effluent is discharged continuously only during a single work shift, or two successive work shifts, at least one composite sample, of sufficient volume to set up the toxicity test, shall be collected;

When a facility retains the wastewater during a work shift, then treats and releases it in a batch discharge, a grab sample shall be collected during the discharge period. Sufficient volume of sample shall be collected for the set up and renewal of the toxicity test during the hours intervening between effluent discharges. Use caution when collecting these samples as wastewater sampling, especially in manholes and enclosed spaces, may involve exposures to vapors of oxygen-depleted atmosphere, requiring suitable precautions.

When a facility discharges wastewater to an estuary only during an outgoing tide, a single grab sample or composite sample (as specified by the Department in the NJPDES permit), of sufficient

volume to set up the toxicity test shall be collected on the outgoing tide. This procedure is repeated for the duration of the toxicity test.

Effluent samples shall be chilled during or immediately after collection for transport to the lab.

Alteration of samples shall be limited to:

Filtration through Teflon® or No. 316 stainless steel screening having a mesh of 2mm or larger. Screening constructed of unplasticized polyethylene or polypropylene may be substituted provided the screens are discarded upon the completion of a bioassay.

Introduction of dry artificial sea salts or hypersaline brine for the purpose of adjusting the effluent test concentration.

Using a dechlorinating agent to reduce the level of chlorine in an effluent sample. Any adjustments made shall be consistent with N.J.A.C. 7:18-9.5(b)6.

All sampling equipment shall be constructed of approved materials in accordance with N.J.A.C. 7:18-7.3 and cleaned in using the methodology in accordance with N.J.A.C. 7:18-7.4(c). Prior to sample collection, containers shall be pre-rinsed with the effluent and then filled, using the specified procedures, so that there is no air space in either the neck or cap.

Unless the purpose of the bioassay is to ascertain the persistence of the toxicity of an effluent, testing shall begin within 24 hours of the collection of an effluent.

6.11.3 The Following Chain of Custody Procedures Shall be Employed in Collecting and Handling Composite or Grab Samples:

Only clean or new containers, previously rinsed with the material being sampled shall be used for taking composite or grab samples.

Tie-on affixed labels with an identification number shall be used for labeling all samples.

After a sample has been collected, the appropriate information as to identity of the sample shall be written on the label and the label affixed. The label shall remain affixed until the test has begun and the surplus has been discarded.

Immediately upon delivery of a sample to the laboratory, the sample collector shall complete the appropriate chain of custody section of the sample report form or chain of custody form.

The chain of custody form shall list at a minimum the following information:

- Sample number;
- Description of samples;
- Specific location of sample collection;
- Identity of person collecting the sample;
- Date and time of sample collection;
- Date and time of custody transfer to laboratory (if the sample was collected by a person other than laboratory personnel);
- Identity of person accepting custody (if the sample was collected by a person other than laboratory personnel);

Date and time of initiation of analysis. Identity of person performing analysis; and Name of the laboratory performing the analyses.